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Shell disease of oysters

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ABSTRACT

SHELL disease is a fungal disease of the shells of oysters of West European waters which has been known since 1887. Initial fungal penetration is marked by spreading areas of white spots inside the shell. Increased penetration irritates the mantle and conchyolin warts are laid down, the shell may become distorted and the adductor muscle detached, and the condition index may fall. Examination of the shell in sections and decalcified preparations shows the presence of a fungus identified as *Ostracoblabe implexa* Bornet & Flahault. This fungus was isolated in pure culture and is described. Experiments to determine the rates of natural infection showed that young oysters are more susceptible to infection and that temperatures in excess of 20°C for two or more weeks are necessary for high infection levels. Some success in obtaining artificial infection by grafting diseased shell and by exposure to the fungus was obtained.

SHELL DISEASE OF OYSTERS

INTRODUCTION

First described in 1887, shell disease is the oldest scientifically recognized pathogenic disease of oysters; it is caused by a fungus which attacks the shells of both the European flat oyster, *Ostrea edulis* L., and the Portuguese oyster, *Crassostrea angulata* (Lmk). The major symptoms are the development of white spots on the inside surfaces of the oyster shell and, in more severe cases, the occurrence of warts. The disease syndrome was not described in full until 1951 (Korringa 1951a) and even this description is brief and without illustration. This lack of information on the subject has led to some misunderstanding in the identification of the disease.

HISTORY OF SHELL DISEASE

THE first recognizable description of shell disease: 'une tache noire et des petits points gris-foncé' was given by the De Montauge brothers at Arcachon, France in 1878. A fuller description, but limited to the severe wart stage of the disease, was given by Giard in 1894 under the name 'maladie du pied'. Within the warts he reported the presence of 'filaments qui peut-être l'état jeune de *Conchelis rosea* Batters'.

In the Netherlands the disease was first noted in 1902 by Hoek. One of the authorities to whom Hoek submitted diseased shell, F. A. C. Went, suggested that the disease might be due to 'fungal threads showing similarity to . . . . *Ostracoblabe*' (Hoek 1902) (see below). Hoek made unsuccessful attempts to culture the mycelium that he could see in the shells.

Shell disease came under suspicion as a possible cause of major oyster mortalities in 1920 in Great Britain (Orton 1924a and b), and in 1930 in France (Voisin 1931), as well as in the Netherlands (Havinga 1931). Only Havinga investigated shell disease to any extent and showed that it did have an effect on the size of the oyster meats. In the light of present knowledge it is, as Orton concluded, unlikely that shell disease was the cause of British, French or Dutch mortalities of this period.

The most detailed investigations of shell disease were carried out by Korringa (1947, 1951a) who described, for the first time, its course from white spot to warting. He also discussed the ecology of the disease, but was unsuccessful in attempts to culture the fungus that he could see in the shells. Unfortunately, Korringa published only a brief resume on shell disease, and more detailed results have yet to be published.

Shell disease has been mentioned in most works concerning oysters and their diseases from 1900 onwards: Hornell (1910); Dantan (1918); Dollfus (1921); Llaguet (1922); Orton (1924a, b); Ranson (1936); Orton (1937); Ranson (1951); Korringa (1951b, 1952); Cole (1956); Yonge (1960); Mackin (1962); Sindermann and Rosenfield (1967); Sindermann (1968); Farley (1968); Johnson and Chapman (1968).

As well as this series of papers on oyster diseases, there exists another series which described fungus-like organisms found inhabiting the shells of marine animals. The most important of these papers is that of Bornet and Flahault (1889), earlier works often tending to be unreliable (Carpenter 1843; Quekett 1852; Rose 1855; Lacaze-Duthiers 1856; Wedl 1859; Kolliker 1859, 1860; Stirrup 1872; Moseley 1875; Duncan 1876; Topsent 1887a, b; Bachmann 1890). Of the ten organisms described by Bornet and Flahault from the inner layers of the sub-nacre of *Ostrea* shells, one, a fungus, *Ostracoblabe implexa* Born. et Flah., is of interest, since its description closely resembles the mycelium seen in the warts of shell disease. The history of the name *Ostracoblabe* is complicated, in that, apparently without adequate reason, it has become considered as a synonym of a marine lichen, an *Arthropyrenia* (Bornet 1891; Bommer 1891a, b; Santesson 1939; Feldmann 1954).

GEOGRAPHICAL DISTRIBUTION OF SHELL DISEASE

OYSTERS of the genera *Ostrea* and *Crassostrea* have a world-wide distribution, but the most important areas of commercial exploitation are in Europe, North America and Japan. There exists only one record of shell disease occurring outside Europe. Durve and Bal (1960) in India found large warts on shells of the Blackwater oyster *Crassostrea gryphoides* (Schlotheim). However it seems unlikely that these warts were due to European-type shell disease. The only certain reports of shell disease are from the Atlantic coast of Europe (Fig. 1) and its two indigenous oysters, *Ostrea edulis* and *Crassostrea angulata*.

NORWAY

Norwegian waters are generally too cold to provide good conditions for shell disease. If diseased oysters or shells were ever to be imported into Norway it is possible that the disease could establish itself in fjord headwaters, which exceed 20°C in summer (Havinga 1931; Gaarder and Bjerkan 1934). There is no shell disease in Norway at present (Gundersen, pers. comm.).

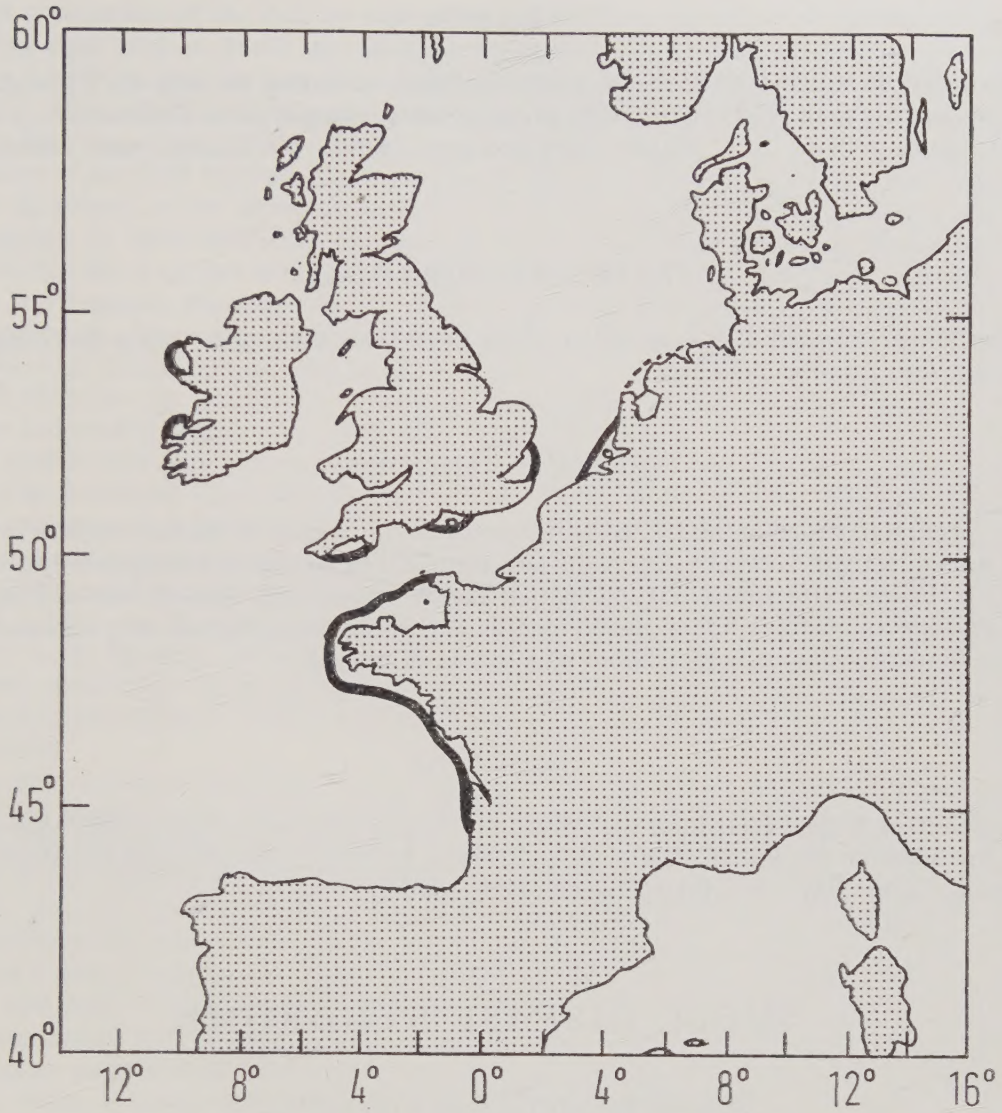

Figure 1 Distribution of shell disease. The areas marked indicate districts with known shell disease.

DENMARK

Shell disease has been recorded by Spärk (1926) among introduced French and Dutch oysters in the Limfjord, but shows no signs of becoming established in Danish waters.

NETHERLANDS

As a result of land reclamation schemes many formerly productive oyster beds have been lost in the Netherlands. The major centres of oyster production were Zeeland and the Oosterschelde, both long-established shell disease areas (Hoek 1902; Havinga 1931; Voisin 1931; Korringa 1951a). The latter author suggested that the widespread use of old shell as cultch to collect spat in the Netherlands was responsible for high rates of disease there.

FRANCE

France is a country in which shell disease is well established, occurring on both the Channel and Biscay coasts (Bornet and Flahault 1889; Giard 1894; Mouliets 1908; Hornell 1910; Dollfus 1921; Llaguet 1922; Voisin 1931; Ranson 1936, 1951; Marteil 1954 and pers. comm.; Le Dantec, pers. comm.; Trochon, pers. comm.).

SPAIN AND PORTUGAL

No published records of shell disease in oysters from these areas exist, and Andreu (pers. comm.) states that he has not seen it in the Vigo area.

UNITED KINGDOM

The disease was first recorded in the United Kingdom in 1949 in imported Brittany oysters in Essex (Cole 1950), and subsequently in native oysters (Cole and Hancock 1956) and in Portuguese oysters (Cole and Waugh 1956). However, Havinga (1931) states that Dutch oysters with shell disease had been exported to the United Kingdom before 1930. Detailed records of shell disease in Britain only commenced in 1956 and these do not really show any indications of a spread of disease.

IRELAND

As in the case of the United Kingdom, shell disease in Ireland was first noted in three-year-old Brittany imports in the Shannon in 1951 (Cole and Waugh 1956). In 1963 it was recorded in native oysters in a restricted area of Clew Bay, Co. Mayo (Duggan, pers. comm.).

SHELL DISEASE SYNDROME

MACROSCOPIC OBSERVATIONS

THE greater part of the inside surface of the normal oyster shell is hard calcite-ostracum (the sub-nacreous layer), but occurring irregularly on the surface of the sub-nacre are deposits of a white porous material; these are known as chalky patches (Korringa 1951c; Durve and Bal 1961).

The first indication of shell disease attack is in the form of small white spots which appear below the surface of the sub-nacreous layer of the shell (Plate 1). It is often difficult to distinguish these spots from small chalky patches which have been covered with healthy shell. At this stage the spots can normally only be identified with certainty as shell disease when they occur on the periphery of areas of more advanced shell disease.

These spots mark the progress of an invading fungal mycelium. The mycelium spreads out through the entire shell, particularly in the inner surface layers. The point at which the mycelium breaks through into the interior develops into a slightly raised area, often with a small central depression. This is the typical shell disease white spot as seen in Plate 2.

Seen from above, the spot has the appearance of a white dot 0.5-3.0 mm in diameter with (in many cases) a minute dark area at the centre. The shade of white of the spot is little different from that of the normal chalky patches, but, unlike the latter, the spots have a definite rough surface relief which can easily be detected. The roughness of the shell disease spot is a clear and decisive identifying character.

The initial penetration of the shell by shell disease is often preceded or accompanied by the presence of shell-boring algae such as *Hyella* spp. which are common shell inhabitants (Bornet and Flahault 1889; Prud'homme van Reine and van Den Hoek 1966). These give a brown or green stain to the affected shell areas (Plate 1). Although these algae penetrate deep into the shell, they have never been observed to cause any harm to the living oyster, unlike the shell disease fungus.

The invasion of the shell continues with the penetration of additional mycelium and the formation of more spots. In young or less intense infestations these spots remain apparent each as a separate and discrete structure. In more advanced infections the spots are so close together that they form a 'cloud' (Korringa 1951a); this is an area with such a density of spots that little or no normal shell is visible between them (Plate 3). Frequently these spot clouds retain the clear white of the young spot, but in extreme cases the shell surface has a brown tint due to the deposition by the mantle of a very thin sheet of conchyolin (Plate 3). The spot clouds may cover a small area in one valve or may be extensive, and entire valves covered with them are not uncommon. Clouds of such dimensions have a much rougher relief than the earlier stages and surface irregularities in excess of 1 mm are common.

Although oysters with such extreme stages of spot are quite common, it is more usual for the secondary wart stage to be developed. The shell disease wart consists of conchyolin; this is the term used to describe the horny proteinaceous material which forms the matrix of lamellibranch shells. Estimates of the total organic content of oyster shells were made by Brandes and Bucholz (1817), Schlofsberger (1856), Chatin and Muntz (1895) and Douville (1936). The conchyolin has been analysed for amino acid composition by Roche *et al.* (1951) and Beedham (1958). In the undiseased oyster conchyolin is normally present in quantity only in the ligament; however, it is also laid down by the mantle in response to strong irritation resulting from penetration of the shell. This irritation can be caused by the penetration of species of *Polydora*—in Europe mainly *P. ciliata* (Johnston), but also *P. hoplura* Claparède and *P. websteri* Hartman (Korringa 1951b).

The perforation of the shell by shell disease fungus is also frequently sufficient to cause a change in the secretions of the mantle of the oyster and the disease thus passes beyond the white spot stage. The form of the conchyolin deposit depends to a great extent on the position of the fungal attack within the shell. At the edges of the shell the normal reaction is an increase in the proportion of conchyolin in the newly-formed shell, giving a hard but brightly coloured sheet, usually bright green although brown tints are common, but when the centre of the disease is away from the edge of the shell the conchyolin tends to be in the form of a wart, generally with very little calcification (Plates 3 and 4). These warts may be 2-4 mm in thickness and may, in places, be partially overlaid with more normal shell. Warts of this type occur throughout the central areas in both valves, but tend to be thicker in the cup valve. Although the warts are the secondary stage of shell disease, they may occur without the prior formation of white spots. Thick warts appear black in colour in contrast to the bright colours of the thinner forms of wart, but this is due solely to their thickness. The initial warts in the adductor attachment area are small rounded black lumps, 1-3 mm across, attached firmly to the shell (Plate 3). With more severe attacks the warts are enlarged, coalescing into one or more knobs in the muscle base (Plate 4). Eventually the muscle attachment may become a raised boss, often with incomplete layers of more normal calcified shell overlying it (Plate 5). This boss-like type of wart is not found outside the muscle attachment and is the type of disease called in France *maladie du pied.*

The progress of shell disease towards its extreme form is marked by the steady extension of spotted and warted areas throughout the shell (Plate 6). The edges of the valves become distorted as a result of uneven development, and proper closure of the shell may become impossible. Heavy warting on the adductor attachment, on one or both valves, reduces the effective area of attachment of this muscle which, although it still covers the warts on the hypostracum, is attached only to the undamaged portions.

Severe disease also develops in the hinge region of the shell, with marked stimulation of shell growth, so that the hinge area develops excessively in relation to the rest of the shell and the oyster has a beaked appearance (Plate 7). The centre hinge region becomes heavily warted and the edges of both valves may become thick and irregular. Much of the wart material on the edges of the valves becomes eroded and the beaked area accumulates mud and bacteria. This form is known in France as *maladie de la charnière* (Plate 5). Frequently, chambers may be cut off in the cup valve.

The combination of the two forms of shell disease damage, *pied* and *charnière*, occurring together in one oyster, is the most severe form of shell disease encountered (Plate 8).

As stated above, the shell disease wart is hard, consisting of black, green or brown conchyolin. Other types of wart or pustule may occur on oysters and this has led to some confusion. The pustules described by Orton (1924a and b) and Mackin (1962) are softer yellow ones deposited on the shell. Yellow pustules are, as shown by Farley (1968), a common feature of many oyster diseases. A pustule occurring on the mantle of the oyster which adheres to the shell and is subsequently covered over with conchyolin may be mistakenly identified as shell disease. However, close examination will always differentiate between the two types of pustule.

MICROSCOPIC OBSERVATIONS

Whilst the living oyster tissues may be examined by means of conventional section cutting techniques, these are unsatisfactory for the examination of diseased shell and wart material. Although examination of sectioned shell material enables some of the characters of the organisms within the shell to be recognized, the optical effects involved in the shell material make detailed observation impossible. The best method for examining structures in shell is by decalcification. The traditional method of decalcifying materials is by the use of dilute acids, for example the Pyreni solution of Bornet and Flahault (1889). More recently, metal-chelating agents have been introduced for use as decalcifiers (Hahn and Reygadas 1951; Sreebny and Nikiforuk 1951; Hillemann and Lee 1953; Schajowicz and Cabrini 1956; Prud'homme van Reine and van Den Hoek 1966). In this investigation the chelate employed was the disodium salt of ethylenediaminetetraacetic acid (EDTA(Na_2)) at 5.5% w/v in distilled water. Although EDTA(Na_2) is considerably slower in action than acid decalcifiers, the pH of the solution employed is near neutrality and very little structural damage is done to the tissues involved.

Chips of oyster shell decalcified with EDTA(Na_2) leave a gelatinous mass which is the organic matrix of the shell. This jelly is easily mounted in the form of a squash preparation and is very suitable for examination with a phase-contrast microscope.

When the shell with the initial white spot stage is examined in the form of a thin section or chip, the white spots are apparent as dark masses, frequently with light centres. Radiating from these spots are fine thread-like structures which have all the appearance of fungal hyphae (Plates 9 and 10). The mycelium is seen to lie in the innermost regions of the shell, and although only vegetative structures have been seen in the shell these are highly characteristic.

The mycelium is very straight, 2 μm wide and varies little in width in any shell (Plate 11); at frequent but irregular intervals it swells to about twice its normal width to form chlamydospore-like structures 4 μm wide by 6 μm long (Plates 11 and 12). In untreated shell it is not possible to discern any structural details within the mycelium. Lateral branches occur at irregular intervals, making an oblique angle with the main axis of the mycelium.

Also visible in the shell are the filamentous thalli of shell-boring algae such as *Hyella* spp., *Gomontia* spp., etc. (Bornet and Flahault 1889; Prud'homme van Reine and van Den Hoek 1966). In fresh shell preparations the green pigmentation of these algae is unmistakable, whilst in older preparations they still cannot be confused with the shell disease fungal mycelium. Penetrations by algal thalli do not produce the aberrant white spot on the inside surface of the sub-nacre.

The same characteristic mycelium is present in the warts, ramifying through the wart right to its surface (Plate 13). No algal thalli have been observed in wart material.

In decalcified diseased shell the fungus is apparent as a fine aseptate colourless mycelium of the same breadth (2 μm) as in fresh material, and shows the same chlamydospore-like structures (Plate 14). Under phase contrast it is apparent that many of the canals seen in the shell and wart are not occupied by living mycelium. In the dead sections of mycelium septa are present (Plate 15), often several in close succession.

Prolonged investigations have failed to show any direct signs, macro- or microscopic, of fungal invasion of the living tissues. As stated previously, only in the most severe stages of shell disease are there any macroscopic signs of damage to the meats. This observation has been repeated in microscopic investigations of mantle tissues from areas overlying shell disease symptoms. In very heavily-diseased oysters, the cells of the mantle epithelium tend to be predominantly long and thin rather than cubical, which is the normal healthy form.

Difficulty was encountered in that there are no adequate accounts of the histology of the oyster mantle. The functions of the three mantle lobes have been investigated (Korringa 1952; Yonge 1960) and these carry out the major function of secreting new shell. The oyster mantle epithelium is little known except in the region of the ligament (Siebert 1913; Trueman 1949, 1950, 1951). A thorough investigation of the mantle in diseased and disease-free oysters has failed to discover any abnormal features in the mantles of diseased oysters beyond the increased proportion of long epithelial cells in certain heavily-infected specimens. In moribund oysters, with extremes of shell disease, a general disorganization of tissues is apparent. This is not limited to the mantle or to parts of the meats directly related to severe shell damage.

ISOLATION AND CULTURE OF THE FUNGUS

KNOWLEDGE of most fungal diseases of marine animals has not passed beyond the simple stage of recognition and description, and rarely has the causative organism been isolated and the disease properly investigated. This is certainly not because such marine fungal diseases are uncommon, since there are many papers and notes describing different ones, e.g. Atkins (1929, 1954a, b); Couch (1942); Ganaros (1957); Sandoz *et al.* (1944); Schwartz (1963). Those diseases which have been investigated have shown that the fungi responsible are likely to be demanding in their requirements and that conventional mycological culture methods are not likely to be successful. In the case of shell disease, Hoek (1902), Havinga (1931) and Korringa (1951a) tried repeatedly to isolate the organism visible in the shell, without success.

In the present study a wide range of media and techniques, conventional and unconventional, was tried without success before a suitable medium was found. Successful isolation was achieved by the following methods. Oysters from Althorne Creek, Essex were thoroughly scrubbed and washed five times in sterile sea water before being opened with a flamed knife. The meats were removed and valves showing advanced shell disease warts were selected. These were washed again in sterile sea water five times, and then placed in sterile wide-mouthed screw top jars. Each jar contained 100 ml of sterile sea water and 0.05 g penicillin G and 0.05 g streptomycin sulphate base (as 0.1 g Crystamycin Glaxo). The jars were incubated at 25°C, laid on their sides so that the half shells were completely covered by sea water. The shells were examined daily and transferred aseptically to a fresh jar at the first sign of bacterial development; usually, several such transfers were necessary in the 5-week incubation period. Within this period, the shell disease warts and some diseased parts of the shell became covered by white wefts of mycelium. Some forty different 'cultures' were made in this manner, and in every case the mycelium was found to be entirely of the shell disease type.

A development of this technique was to excise small pieces of wart from diseased shells with a sterile knife and wash them five times in sterile sea water before transferring them aseptically to test tubes containing 10 ml of sterile sea water and 0.4 g Crystamycin (Glaxo). This concentration of the antibiotics is sufficient to suppress bacterial development. The test tubes were maintained at 25°C and examined weekly; mycelial development was apparent at about 2 weeks in most cases. The fungus may be maintained on the wart tissue for considerable periods (up to 6 months at least); it may also be transferred on to 'bait' and so maintained indefinitely. Several different conventional baits have been tried—hemp seeds, grass, and nail pairings—in sea water; of these, only hemp seeds and nail pairings were suitable, but sterilized wart or oyster ligament are equally good. Transfer is effected by introducing the bait into the culture for 2 weeks and then removing it to a fresh test tube of sterile sea water.

Plate 1 Early shell disease white spots (arrowed). The surrounding dark patches (a) are caused by algae penetrating the shell.

Plate 2 Close-up of white spots. Many of the spots may be seen to have dark centres (arrowed).

Plate 3 This oyster shows spot clouds (S). To the right and left of the adductor muscle the shell is being covered with a dark layer of thin conchyolin (C). In the adductor attachment areas are small round knobs of conchyolin (arrowed).

Plate 4 This oyster has a large conchyolin wart (W) on the adductor muscle attachment, together with extensive conchyolin sheets (C) radiating to the margins of the valve.

Plate 5 The adductor muscle attachment of this oyster has been converted largely into a raised boss of conchyolin which has subsequently become partially covered with more calcified shell layers (W). The shell also shows a very well-developed spot cloud (S) and small warts developing near the hinge (*maladie de la charnière*) (arrow).

Plate 6 In this oyster, the development of the disease (W) has resulted in considerable distortion of one margin of the valve.

Plate 7 Severe distortion and thickening of the shell margin (*maladie de la charnière*) occurred in this oyster as a result of strong disease activity along the edges and hinge of the valve (arrows). The adductor is warted (W) but there are relatively few white spots in the interior of the valve (S).

Plate 8 This valve represents an extreme example of shell distortion. The actual space available for the meats in this shell is very restricted and the adductor (A) is so diseased that the oyster could be opened with the fingers.

Plates 9 and 10 Sections of diseased shell at the white spot stage. The dark patches are the white spots (S) from which fine mycelium (m) radiates ($\times 60$).

Plate 11 Section of diseased oyster shell showing typical features of the mycelium within the shell. The mycelium is straight and one oblique branch is shown. The arrow indicates a chlamydospore-like structure. This oyster was infected artificially ($\times 150$).

Plate 12 High-power view of the chlamydospore-like swelling shown in Plate 11 ($\times 300$).

Plate 13 Section of shell disease wart showing mycelium with a chlamydospore-like structure ($\times 600$).

Plate 14 Decalcified preparation of diseased shell to show living mycelium with chlamydospore-like structure (arrowed) ($\times 900$).

Plate 15 Decalcified preparation showing a short piece of dead mycelium with a septum ($\times 900$).

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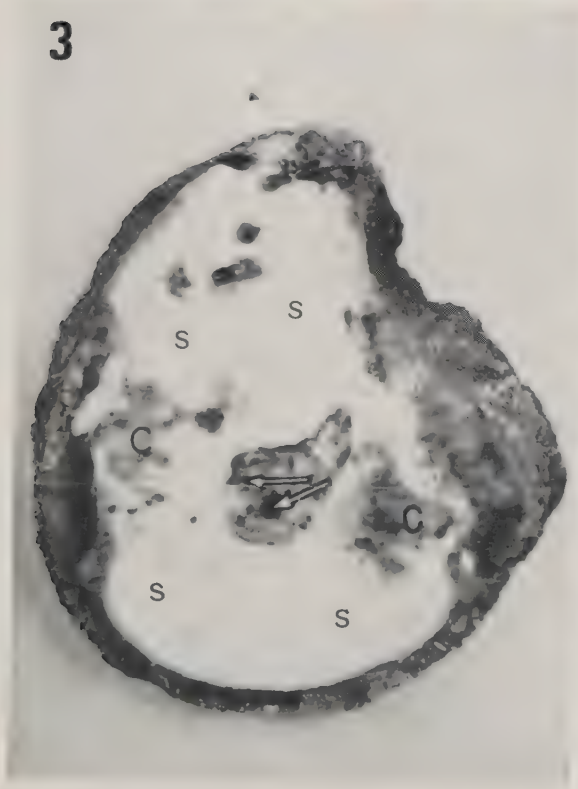
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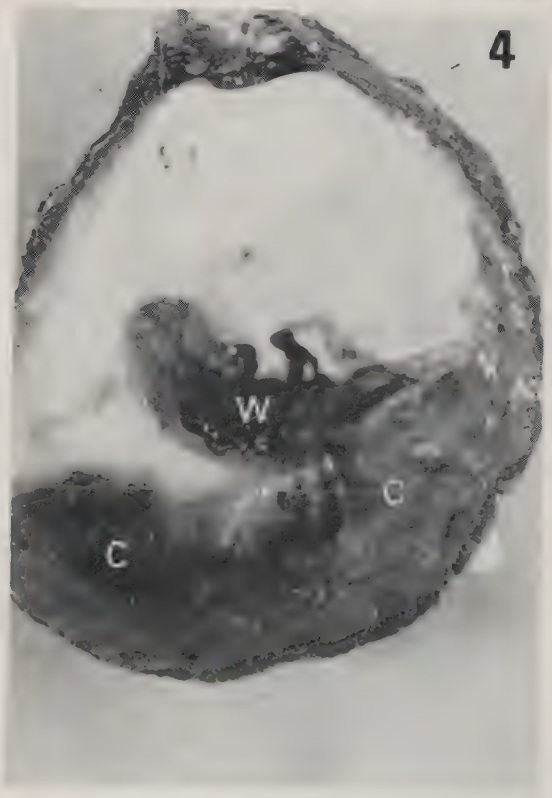
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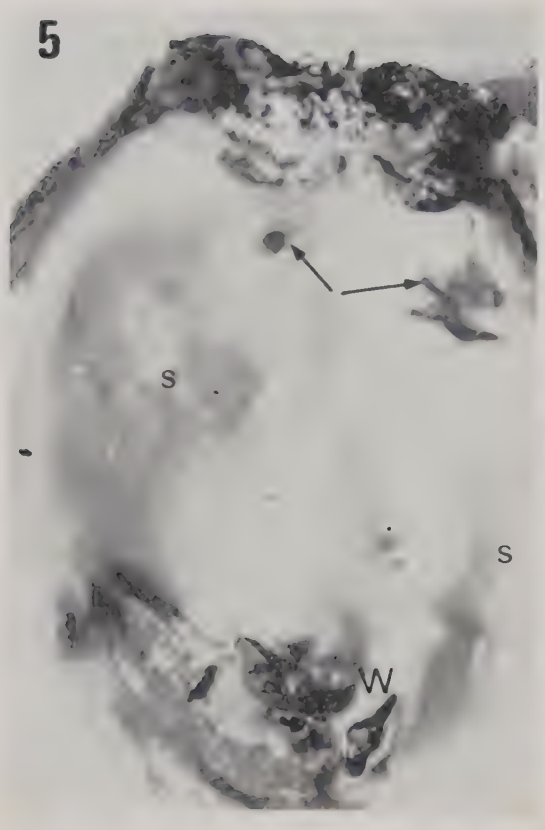
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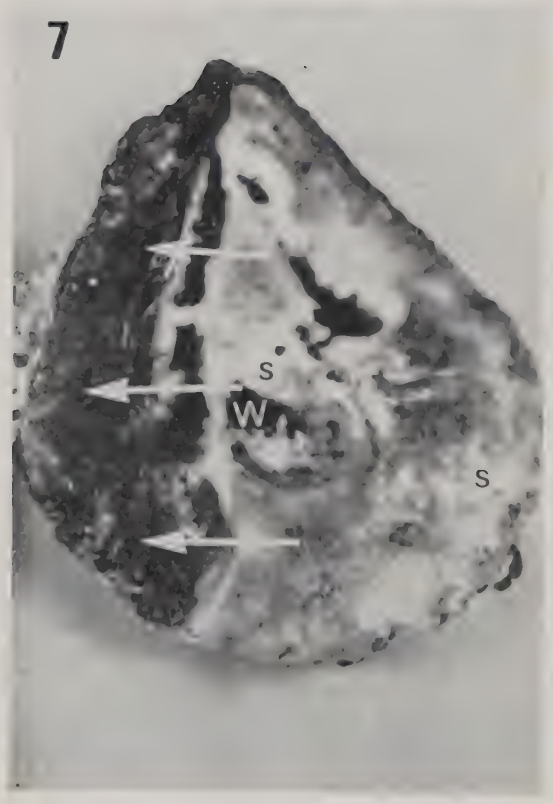
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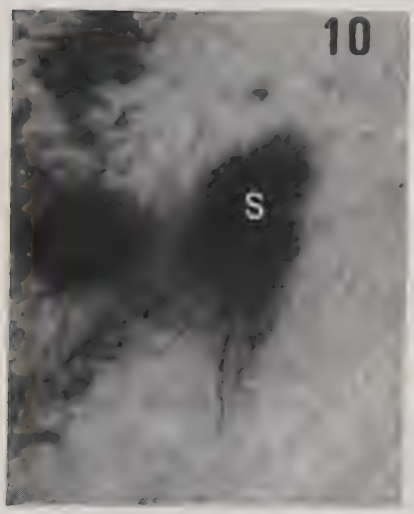
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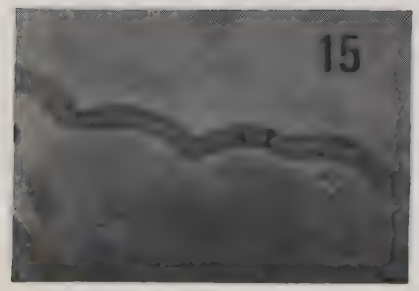
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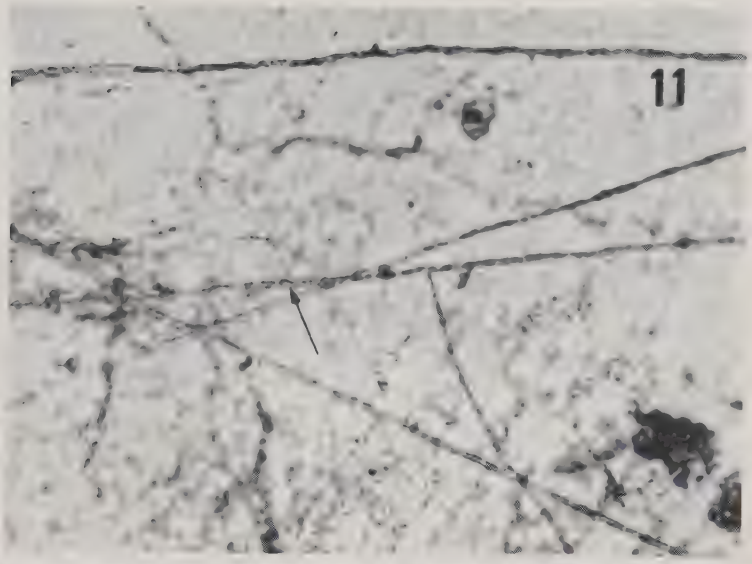
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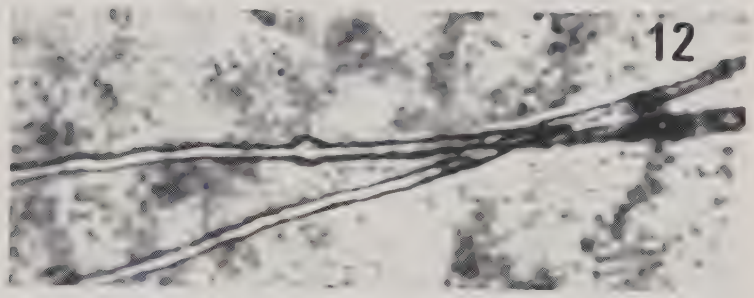
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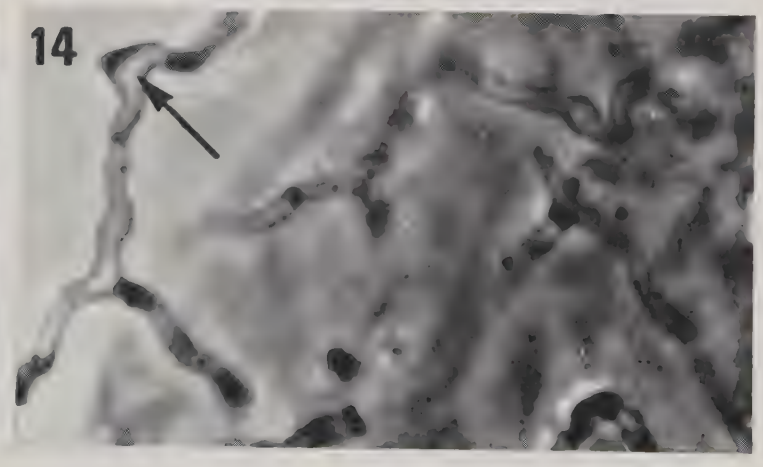
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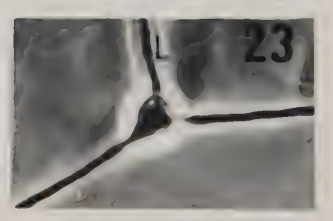
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Plates 16, 17 and 18 Pure cultures in yeast/peptone broth of the shell disease fungus *Ostracoblabe implexa* (Plate 16 ×50; Plate 17 ×100; Plate 18 ×250).

Plate 19 Older mycelium showing dense cytoplasm (×500).

Plates 20, 21 and 22 Photomicrographs of mycelium grown at 5°C for 3 weeks. The mycelium is more vacuolate, septa develop (Plate 20) and the pro-chlamydospore have formed true chlamydospores (all photographs ×1 250).

Plate 23 This figure shows formation of a lateral branch (L) from a pro-chlamydospore after starvation (×250).

With the organism isolated in bait culture, preliminary growth experiments were undertaken to determine if it would grow on artificial media. Since the organic content of the oyster shell is low it is apparent that the fungus must be capable of growing under conditions of low nutrient availability. With this in mind, a low-nutrient seawater broth was selected. This medium, designated YP1, was composed of:

Yeast extract (Oxoid L20)	1.0 g
Peptone, mycological (Oxoid L40)	1.0 g
Sea water to	1 000 ml

50 ml of medium per 250-ml Erlenmeyer flask.

Wart material, incubated in a test tube as described above, was transferred into this medium by emptying the contents of the tube into the flask. By making the transfer in this way the antibiotics in the test tube were also transferred, reducing the chances of bacterial contamination in the initial artificial culture. The flasks were then incubated at 25°C. Growth was good in this medium and it has subsequently been used as a standard medium.

Stock cultures of the various isolates have also been maintained on conventional agar slopes using yeast/peptone seawater agar. The atmosphere over the slopes is kept saturated by the presence of not less than 3-4 ml of sea water at the bottom of the tube. Such cultures remain viable for up to two years.

Although the majority of cultures have been obtained from warted material, the fungus has also been isolated from diseased shell at the spot stage. Shell chips placed in sea water plus penicillin/streptomycin do not produce visible amounts of mycelium. Chips inoculated directly into yeast/peptone agar, with penicillin/streptomycin added, frequently produce actively growing shell disease mycelium.

As stated above, the shell disease fungus has not been observed in the living oyster tissues, although 100 attempts have been made to isolate it from mantle and other tissues, employing the techniques described above. No organism resembling that seen in the shell has ever been isolated from the meats nor has any other fungus been isolated from them with any regularity.

DESCRIPTION OF ISOLATE

The mycelium is colourless, and under phase contrast it may be seen that it contains a dense, finely granular, protoplasm. It is 2 μm wide and very straight (Plates 16–19). In the young mycelium no septa have been observed except where they cut off sections in which obvious physical damage has occurred. With increasing age the protoplasm becomes increasingly granular and septa may be formed.

The mycelium bears ovoid swellings (4 × 6 μm) at frequent but irregular intervals. They are intercalary or terminal (Plates 18 and 19). They are not in any way cut off from the mycelium and cannot therefore be described as chlamydospores, which are thick-walled resting spores. However, in ageing cultures septa may be formed, and in mycelium kept at low temperatures nearly all swellings increase in size and develop septa and thicker walls (Plates 20–22); these structures may be described as chlamydospores. Since the mycelial swellings are capable of developing into chlamydospores proper, under appropriate conditions, the term *pro-chlamydospore* is used for the swellings.

Growth in different media does not exhibit many major morphological variations. Starved mycelium is thinner (1 μm wide) and mycelium grown in a rich nutrient medium (4 g yeast/peptone per litre) is thicker (3 μm wide). When starved mycelium is inoculated into normal media, frequently new lateral branches are formed from the pro-chlamydospores (Plate 23); this does not occur in normal mycelium.

No sexual reproductive structures have been observed. *Ostracoblabe implexa* Bornet and Flahault (1889) closely resembles shell disease mycelium. The diagnosis provided by Bornet and Flahault is sufficient to permit easy recognition and therefore the valid name of the shell disease fungus is *Ostracoblabe implexa* Bornet and Flahault (1889). There is some evidence that this sterile mycelium may have as an asexual reproductive state a free-floating biflagellate sporangial fungus (Jones and Alderman 197-).

OCCURRENCE OF SYMPTOMS ON THE SHELL

DURING the course of this investigation records have been kept of the severity and position of disease symptoms on oyster shells from several sites. Table 1 lists the percentage occurrence of different degrees of shell disease in a number of oysters from Althorne Creek, River Crouch, Essex. For the purpose of recording them, the stages of shell disease were divided arbitrarily into six classes as follows:

Class 0—No disease
Class 1—One to many white spots
Class 2—Spots forming a cloud
Class 3—Slight warting and/or extreme spotting
Class 4—Heavy warting, meats possibly affected
Class 5—Extreme disease, meats visibly affected.

The figures given in Table 1 show that in Althorne Creek 68% of the oysters are infected, and of these 28% (Classes 4 and 5) may be expected to be unsuitable for sale due to their unpleasant appearance and weak or watery meats.

Table 1 Percentage occurrence of degrees of shell disease in samples of oysters from Althorne Creek, Essex

Date	Class						Total number of oysters
	0	1	2	3	4	5	
24 Feb. 1966	10	14	38	10	5	24	24
20 Aug. 1966	33	17	3	10	24	10	30
16 Sep. 1966	36	26	6	12	12	8	60
4 Oct. 1966	48	4	8	6	11	20	45
18 Jan. 1967	28	19	19	14	14	6	333
16 Feb. 1967	39	12	16	18	9	9	50
24 Apr. 1967	40	20	10	17	13	0	30
1 June 1967	52	12	8	12	12	4	25
6 June 1967	10	30	27	23	10	0	30
18 Aug. 1967	42	24	33	7	5	0	43
19 Sep. 1967	30	40	8	16	0	8	26
31 Oct. 1967	10	27	20	20	20	3	30
23 Nov. 1967	40	18	11	11	10	0	192
26 Jan. 1968	22	33	19	14	11	0	36
9 Feb. 1968	9	29	24	18	21	0	34
Total number of oysters	312	191	159	130	118	77	987
Mean % in each class	32	20	16	13	12	8	
% of diseased oysters	–	28	24	19	17	11	

Fig. 2(i) illustrates the sectors of the oyster shell used for recording the distribution of symptoms. Fig. 2(ii) shows, in the form of a *rose* diagram, the frequency of occurrence of disease in each sector of the shell, the length of each arm of the rose representing the proportion of the total number of infections recorded in the corresponding area. The diameter of the central discs represents infections on the adductor base. The diagram shows clearly that disease occurs more frequently in some areas of the shell than in others. It seems possible that these areas are, as a result of the shape of the oyster, those most consistently in contact with the ground or other shells.

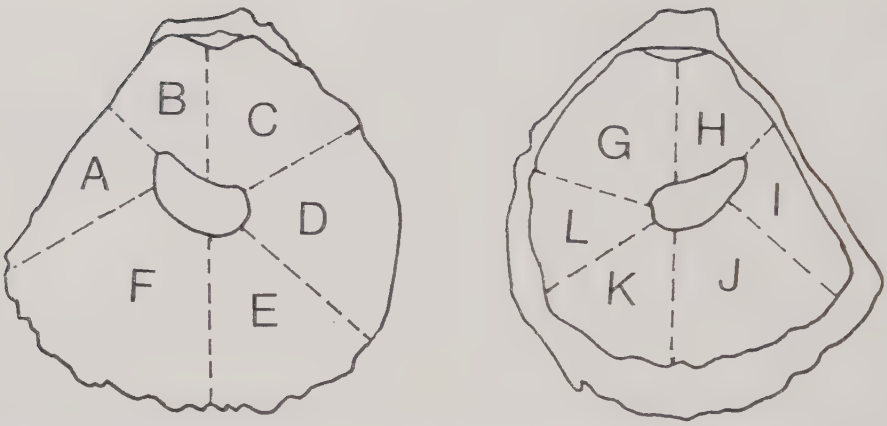
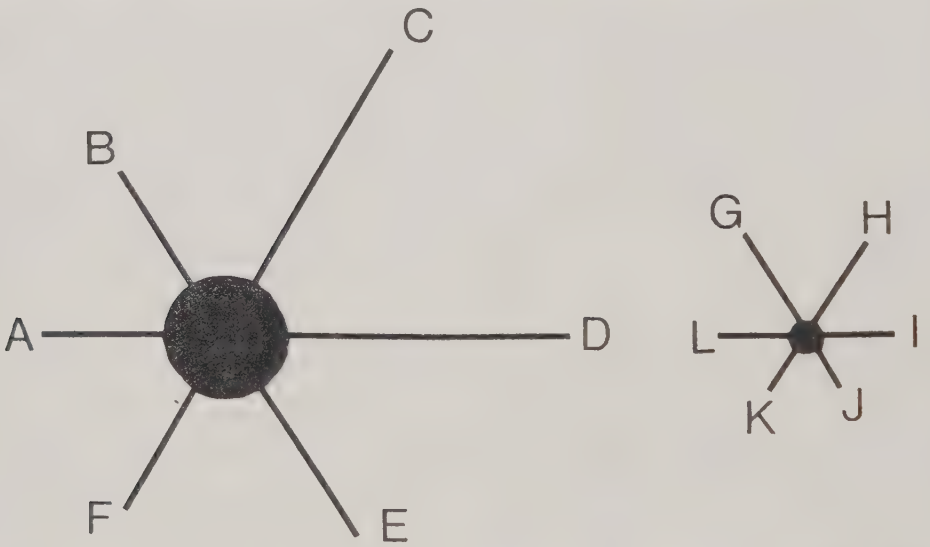


Figure 2(i) Diagram showing the shell sectors used in shell disease investigations.



(*ii*) Rose diagram showing the frequency of infection for the different areas of the oyster shell. The length of the arm indicates the proportion of disease in that sector. The central discs represent infections of the adductor attachment. (Sector D had 179 infections).

ARTIFICIALLY-INDUCED INFECTIONS

Korringa (1951a) stated that by attaching a piece of diseased shell to a disease-free oyster he was able to infect the oyster. Unfortunately he gave no details of his methods, and it was felt that it was necessary to repeat this work under defined conditions.

Using a dental workshop drill, small (0.5 mm^2) pieces of diseased shell at the white spot stage were taken. Healthy one-year-old Norwegian oysters, known to be free of shell disease, were used as recipients. A small area of the recipient oyster's shell was ground flat with dental abrasives and the implant attached by a ring of ciment-fondu. The oysters were placed in a recirculating seawater system at 25°C for 6 months and were fed from bulk cultures of *Isochrysis galbana* Parke (Walne 1966). Two hundred oysters were used in this experiment and another hundred were kept as controls in separate tanks, but using the same intake of sea water. These controls were maintained to establish whether significant quantities of infective material were present in the water.

At the end of 6 months none of the control oysters were infected but four of the experimental oysters showed white spots at the site of the graft. On examination these spots were found to be related to infesting shell disease mycelium. No mycelium was found in control oyster shells.

The low rate of infection achieved is probably a direct result of the techniques used; factors such as overheating by the dental drill or abrasive, and toxic and intrusive effects of the cement militated against a high rate.

Similar difficulties were encountered in using mycelium to produce infection. Out of 40 one-year-old Conway oysters, kept for 4 months at 25°C with fresh mycelium added at 0, 2 and 8 weeks and with frequent changes of water, only one resulted in one white spot infection. The major obstacle in this experiment was the difficulty of keeping down the numbers of protozoa which grazed on the very slow-growing mycelium. These results are regarded as preliminary indications, and with improved experimental techniques and conditions it is hoped that better results may be obtained.

INFECTION UNDER NATURAL CONDITIONS

ALTHOUGH there are many records of the occurrence of shell disease, these are not sufficiently continuous to give information about conditions which bring about infection in nature. Therefore, at sites in Essex in 1966 and 1967, experiments were carried out which show clearly that temperature plays a major role in determining the rate of infection.

Imported one-year-old Norwegian oysters were sampled to confirm that they were disease-free and the rest were placed in trays at three sites in the Crouch/Roach system: Althorne Creek, Paglesham Pool, and Shop Laying. In 1966, of 1 050 oysters examined 1.5% developed shell disease that year, whilst in the following year, 1967, of 450 examined 4% were diseased. Oysters imported in 1967 were also laid in trays at the same sites in that year; of these, 25% developed disease. These results are shown in Fig. 3, and Fig. 4 shows the mean weekly temperature of the water during the summer for 1966 and 1967 in the River Crouch. Clearly, 1967 was the warmer year and infections were much higher. Korringa (1951a) suggests that young oysters are more susceptible to shell disease. The results described above would tend to confirm Korringa's observations, since the 1966 oysters were two years old in 1967 and were then only 4% infected, in contrast to the 25% of the one-year-old 1967 oysters.

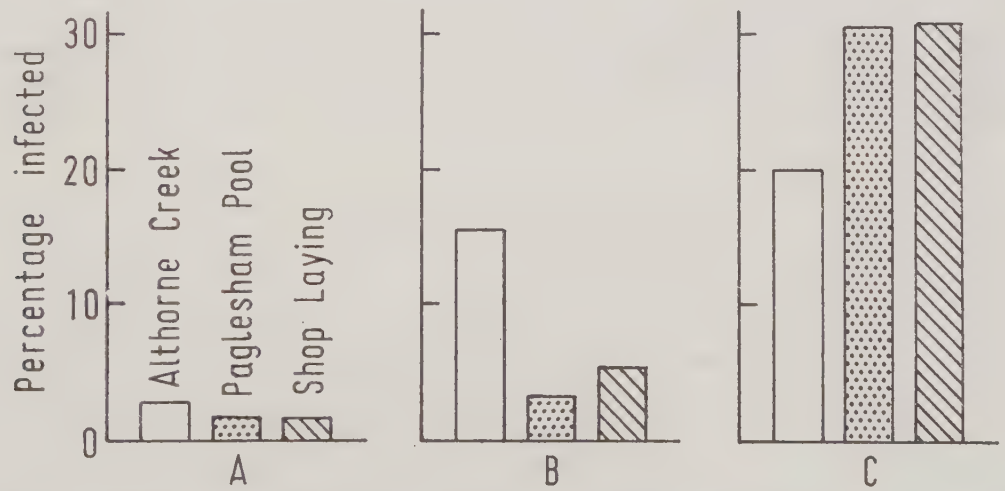


Figure 3 Rates of natural infection of oysters at three sites in the Rivers Crouch and Roach in Essex.
A. Percentage infection of one-year-old Norwegian oysters in 1966.
B. Percentage infection of the same oysters (now two years old) in 1967.
C. Percentage infection of one-year-old Norwegian oysters in 1967.

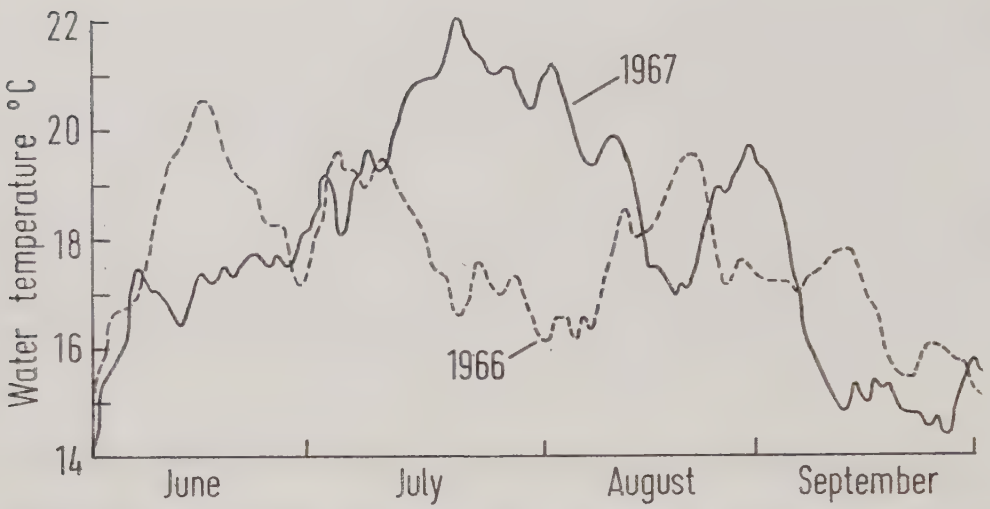


Figure 4 Summer water temperature records for Black Point, River Crouch, during 1966 and 1967.

QUANTITATIVE EFFECTS OF DISEASE ON OYSTER MEATS

Havinga (1931) showed that shell disease probably had an effect on the wet weight of oyster meats. Using the well-known condition index as used by Ray *et al.* (1953) in connection with *Dermocystidium* disease in America, the effect of infection by shell disease on the meats of *Ostrea edulis* has been determined. The condition index (C.I.) is derived from the formula

$$\text{C.I.} = \frac{\text{Meat weight (dry)}}{\text{Volume between valves}} \times 100.$$

Five hundred oysters from Althorne Creek were examined. All were carefully scrubbed clean of external debris. The volume between the two valves was determined gravimetrically, and the meats were dried at 50°C for 2 days and at 100°C for 2 days. The shells were examined for disease and classed according to the arbitrary system described previously on page 11. Fig. 5 is a histogram showing the arithmetic means of the condition indices for all classes of diseased oysters. Classes 3, 4 and 5 represent oysters which have progressed to the warting stage; it is clear that these are markedly weakened by the disease.

CONCLUSIONS

It has been shown that shell disease is caused by a phycomycetous fungus, *Ostracoblabe implexa* Born. et Flah. which attacks the oyster shell and which by causing irritation induces the formation of abnormal shell.

The disease, which is identical with *maladie du pied* and *maladie de la charnière*, is not at present of any major economic importance and is severe only on certain oyster beds where relatively high water temperatures occur. This is in agreement with the temperature requirements of the isolated fungus (Alderman and Jones 197-), which has an optimum for growth of 30°C and grows little at 15°C. Temperatures above 20°C produce marked increases in growth of *Ostracoblabe implexa*, and levels of shell disease infection are high only when water temperatures are above 20°C. Since the optimum for *O. implexa* is 30°C, it would be very unwise to introduce oysters from known infected areas to warmer climates.

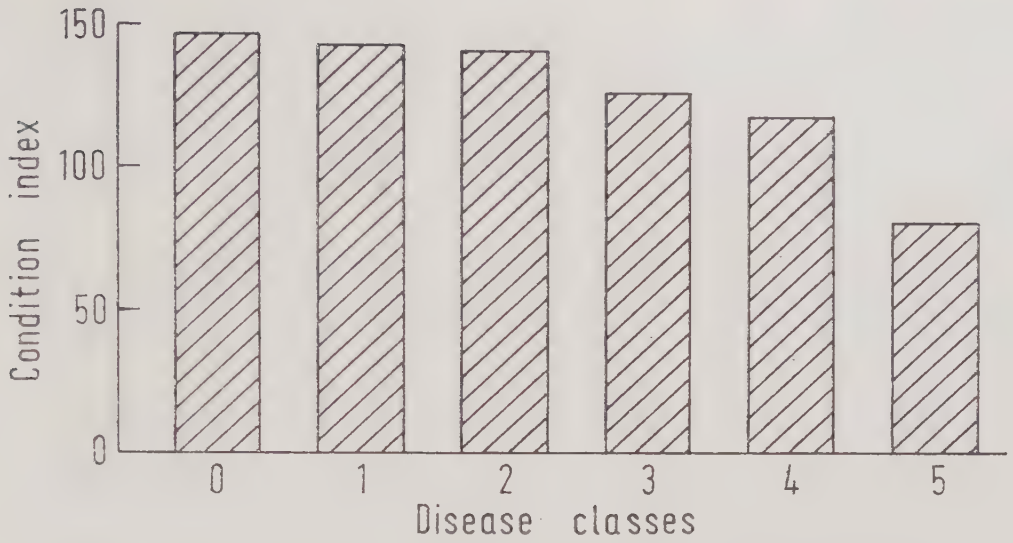


Figure 5 Mean condition indices for oysters in each shell disease class.

During the present investigation no major search has been undertaken to discover whether shellfish other than oysters can be infected by the disease. The shells of other molluscs which have been examined have not shown any disease symptoms, nor has it been possible to trace any published report of damage to other shellfish. It would seem that oysters are the only susceptible organisms. Why this should be so is not clear, but it is possibly related both to the occurrence of oysters in dense communities, i.e. in beds, and to their shell structure. It is also possible that oysters are the only molluscs which react to shell penetrations and irritations in a way that results in shell disease symptoms.

The available data indicate that shell disease is not normally a major hazard in Britain and France. It is rather a minor, but recurring nuisance which could, without forewarning, cause major damage in a warm summer. A year with a warm spring and summer is likely to produce a high rate of infection. The strong possibility exists, therefore, that in an area in which shell disease is present, as a result of the effect of temperature on the growth of the fungus, a good spatfall will also have a high percentage of disease.

It has been shown that shell disease can be transmitted by water and by direct contact with diseased material. In infection by contact the agency of infection will be the mycelium growing from one shell to another.

It remains to consider possibilities of ameliorating the effects of shell disease: is it possible to cure it or to prevent it? Since shell disease is caused by a fungus growing in the shell, the possibilities of curing infected oysters are low. It has been suggested that a suitable technique would be to immerse diseased oysters in saturated brine for a few hours. Apart from the difficulty of ensuring adequate penetration by the brine, *Ostracoblabe implexa* will grow in salt concentrations three times that of normal sea water (Alderman and Jones 197–); brine treatment is therefore unlikely to have any particular value. Korringa (1951a) used mercuric chloride solution as an oyster wash. The young oysters which he used remained closed during the immersion and were unaffected, but the fungus living in the shell was killed. Since it is difficult to ensure adequate penetration, and mercuric chloride is a hazardous chemical to use, it would be unwise to recommend its general use. If there were any possibility of mercuric chloride getting into the creeks and rivers as a result of careless handling, the resulting damage might far outweigh losses resulting from shell disease. Similar objections apply to dipping spat-collecting tiles in mercuric chloride, and as the majority of commercial fungicides are mercury or sulphur salts, similar dangers apply to their use. Although careful use might eliminate shell disease for a while, there is far too great a risk of causing widespread pollution.

In the long run, prevention may be far more efficient and far safer than cure. It is immediately obvious that the only effective way of removing shell disease from an area is to clear the entire area of all oysters and other shellfish, living or dead, and then to relay with oysters from a disease-free source. The economics of clearing an oyster bed of *Crepidula* have been discussed by Cole (1956), and similar criteria will apply to clearance of beds of shell disease. If complete clearance is projected, it is necessary to take into consideration the condition of nearby beds. If these are in poor condition and badly diseased, then localized clearance may be ineffective.

If the policy of complete systematic clearance is economically impracticable, there still remain a number of ways in which shell disease may be reduced. Where possible, a policy of deliberate and selective dredging on the most diseased areas should be followed. If no oysters are relaid on such an area until it has been cleared, the disease may be drastically reduced. This policy is likely to be even more effective if all shell material brought up in the course of dredging is disposed of on land, and is not returned to the water. On no account should oysters from a heavily-diseased bed be transferred to a lightly-diseased area, even if conditions on that bed are unfavourable to shell disease. It is also important that the bottom debris brought up in the dredges from a diseased bed should be returned only to that bed (if it cannot be disposed of on land) and that it should not be dumped overboard while passing over other, possibly undiseased, beds. The practice of using shram, which could harbour the fungus, to line oyster pits is inadvisable in areas where shell disease is present.

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REFERENCES

Alderman, D. J. and Jones, E. B. G. (197–). The physiological requirements of two marine Phycomycetes, *Althornia crouchii* and *Ostracoblabe implexa*. *Trans. Br. mycol. Soc.* (in press).

Atkins, D. (1929). On a fungus allied to the Saprolegniaceae found in the pea-crab *Pinnotheres*. *J. mar. biol. Ass. U.K.*, **16,** pp. 203–219

Atkins, D. (1954a). Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n.sp. infecting certain invertebrates. *J. mar. biol. Ass. U.K.*, **33,** pp. 613–625.

Atkins, D. (1954b). A marine fungus *Plectospira dubia* n.sp. (Saprolegniaceae) infecting crustacean eggs and small crustacea. *J. mar. biol. Ass. U.K.*, **33,** pp. 721–732.

Bachmann, E. (1890). Die Beziehungen der Kalkflechten zu ihrem Substrat. *Ber. dt. bot. Ges.*, **8,** pp. 141–145.

Beedham, G. E. (1958). Observations on the non-calcareous component of the shell of the Lamellibranchia. *Q. Jl microsc. Sci.*, **99,** No. 3, pp. 341–357

Bommer, C. (1891a). Note sur le *Verrucaria consequens* Nyl. *Bull. Soc. belge. Microsc.*, **16,** pp. 79–99.

Bommer, C. (1891b). Un champignon pyrénomycète se développant sur le test des Balanes. *Bull. Soc. belge. Microsc.*, **16,** pp. 151–154.

Bornet, E. (1891). Note sur *l'Ostracoblabe implexa* Born. et Flah. *J. Bot., Paris*, No. 23, pp. 397–400.

Bornet, Ed. and Flahault, Ch. (1889). Sur quelques plantes vivant dans le test calcaire des mollusques. *Bull. Soc. bot. Fr.*, Ser. 2, 11, pp. CXLVII–CLXXVI. (Congrès de Botanique Tenu à Paris en août 1889).

Brandes, R. and Bucholz, C. F. (1817). Analyse der Austernschalen (Ostrea edulis). *Neues Journ. d. Pharm.*, **II,** pp. 204–214.

CARPENTER, W. B. (1843). General results of Microscopic Inquiries into the minute structure of the skeletons of mollusca, crustacea and echinodermata. *Ann. Mag. nat. Hist.*, **12,** No. 79, pp. 377–390.

CHATIN, A. and MUNTZ, A. (1895). Chimie animale.—Analyse des coquilles d'huîtres. *C. r. Hebd. Séanc. Acad. Sci., Paris*, **120,** pp. 531–534.

COLE, H. A. (1950). Shell disease in relaid French oysters. *Nature, Lond.*, **166,** pp. 19–20.

COLE, H. A. (1956). *Oyster Cultivation in Britain.* Min. of Agric. Fish. and Food. H.M.S.O., London, 43 pp.

COLE, H. A. and HANCOCK, D. A. (1956). Progress in oyster research in Britain 1949–54, with special reference to the control of pests and diseases. *Rapp. P.–v. Réun. Cons. perm. int. Explor. Mer*, **140,** Part III, Chapter 6, pp. 24–29.

COLE, H. A. and WAUGH, G. D. (1956). Shell disease in Portuguese oysters. *Nature, Lond.*, **178,** p. 422.

COUCH, J. N. (1942). A new fungus on crab eggs. *J. Elisha Mitchell scient. Soc.*, **58,** pp. 158–162.

DANTAN, J. L. (1918). La biologie des huîtres et l'industrie ostréicole. *Bull Inst. océanogr. Monaco*, No. 341, pp. 1–16.

DOLLFUS, R. F. (1921). Resumé de nos principales connaissances practiques sur les maladies et les ennemis de l'huître. *Notes Mém. Off. scient. tech. Pech. marit.*, No. 7, 46 pp.

DOUVILLE, H. (1936). Le test des lamellibranches: sa formation dans *l'Ostrea edulis. C. r. Lebd. Séanc. Acad. Sci., Paris*, **203,** pp. 965–968.

DUNCAN, P. M. (1876). On some Thallophytes parasitic within recent Madreporaria. *Proc. R. Soc.*, **25,** pp. 238–257.

DURVE, V. S. and BAL, D. V. (1960). Shell disease in *Crassostrea gryphoides* (Schlotheim). *Curr. Sci.*, **29,** No. 12, pp. 489–490.

DURVE, V. S. and BAL, D. V. (1961). Some observations on shell deposits of the oyster *Crassostrea gryphoides* (Schlotheim). *Proc. Indian Acad. Sci.*, **54,** pp. 45–55.

FARLEY, C. A. (1968). *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster *Crassostrea virginica. J. Protozool.*, **15,** No. 3, pp. 585–599.

FELDMANN, J. (1954). Inventaire de la flore marine de Roscoff. Algues, Champignons, Lichens and Spermatophytes. *Trav. Stn biol. Roscoff*, **5,** Supplément 6, p. 136.

GAARDER, T. and BJERKAN, P. (1934). *Osters og osterskultur i Norge.* John Griegs, Bergen, 96 pp.

GANAROS, A. E. (1957). Marine fungus infecting eggs and embryos of *Urosalpinx cinerea. Science*, **125,** p. 1194.

GIARD, A. (1894). Sur une affection parasitaire de l'huître (*Ostrea edulis* L.) connue sous le nom de "maladie du pied". *Bull. Pech. marit.*, **11,** pp. 459–462

HAHN, F. L. and REYGADAS, F. (1951). Demineralization of hard tissues. *Science*, **114,** pp. 462–463.

HAVINGA, B. (1931). Contribution to the knowledge of oyster diseases. Question No. 17. La pathologie de l'huître. VII[e] Congrès International d'Aquiculture et de Pêche. (Paris, 1931). Groupe I, Section 8, pp. 1–9.

HILLEMANN, H. H. and LEE, C. H. (1953). Organic chelating agents for decalcification of bones and teeth. *Stain Technol.*, **28,** No. 6, pp. 285–287.

HOEK, P. P. C. (1902). *Rapport over de oorzaken van den achteruitgang in hoedanigheid van de Zeeuwsche oester.* s'Gravenhage, Ministerie van Waterstaat, Handel en Nijverheid, 168 pp., pp. 82–83.

HORNELL, J. (1910). The practice of oyster-culture at Arcachon and its lessons for India. *Madras Fish. Bull.*, **11,** No. 5, pp. 1–90.

JOHNSON, P. T. and CHAPMAN, F. A. (1968). An Annotated Bibliography of Pathology in Invertebrates other than Insects. Burgess Pub. Co., Minneapolis, 322 pp.

JONES, E. B. G. and ALDERMAN, D. J. (197–). *Althornia crouchii* gen. et sp. nov., a marine biflagellate fungus. *Nova Hedwigia* (in press).

KOLLIKER, A. (1859). On the frequent occurrence of vegetable parasites in the hard structures of animals. *Ann. Mag. nat. Hist.*, Third Series, **4,** pp. 300–303.

KOLLIKER, A. (1860). On the frequent occrurence of vegetable parasites in the hard tissues of the lower animals. *Q. Jl microsc. Sci.*, **8,** pp. 171–188.

KORRINGA, P. (1947). Les vicissitudes de l'ostréiculture Hollandaise élucidées par la science ostréicole moderne. *Ostréiculture, Cultures Marines*, **16,** No. 3, pp. 3–9.

KORRINGA, P. (1951a). Investigations on shell-disease in the oyster, *Ostrea edulis* L. *Rapp. P.-v. Rèun. Cons. perm. int. Explor. Mer*, **128,** Part 2, pp. 50–54.

KORRINGA, P. (1951b). The shell of *Ostrea edulis* as a habitat. *Archs néerl. Zool.*, **10,** pp. 32–152.

KORRINGA, P. (1951c). On the nature and function of "chalky" deposits in the shell of *O. edulis* L. *Proc. Calif. Acad. Sci.*, Ser. 4, **27,** pp. 133–158.

KORRINGA, P. (1952). Recent advances in oyster biology. *Q. Rev. Biol.*, **27,** pp. 266–308 and 339–365.

LACAZE-DUTHIERS, H. (1856). Organisation du dentale. *Ann. des sc. nat. 4e ser., Zoologie*, **5,** pp. 331–352.

LLAGUET, B. (1922). L'ostréiculture dans le Bassin d'Arcachon. *Bull. Stn biol. Arcachon*, **1922,** pp. 71–94.

MARTEIL, L. (1954). Note sur l'ostréiculture en Bretagne-Sud. *Sci. Peche*, No. 16, pp. 4–6.

MACKIN, J. G. (1962). Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publs Inst. mar. Sci. Univ. Tex.*, Vol. 7 for 1961 (pub. 1962), pp. 132–229.

DE MONTAUGE, frères (1878). Etudes practiques sur les ennemis et les maladies de l'huître dans le bassin d'Arcachon. *Actes. Soc. Linné. Bordeaux*, **32,** 4e ser. II, pp. 217–245.

MOSELEY, H. N. (1875). On the structure and relations of certain corals. *Proc. R. Soc.*, **24,** pp. 59–70.

MOULIETS, L. (1908). L'ostreiculture, dans le bassin d'Arcachon. *Bull. Soc. cent. Aquic. Peche*, **20,** pp. 171–178.

ORTON, J. H. (1924a). An account of investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921. Part 1. Report. *Fishery Invest., Lond.*, Ser. 2, **6,** No. 3, 199 pp.

ORTON, J. H. (1924b). An account of investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921. Part II. Appendix A. Interim report on oyster mortality investigations, 1920. *Fishery Invest., Lond.*, Series 2, **6,** No. 4, pp. 3–14.

ORTON, J. H. (1937). *Oyster Biology and Oyster-Culture.* Edward Arnold & Co., London. 211 pp. (pp. 171–172). Buckland Lecture for 1935.

PRUD'HOMME VAN REINE, W. F. and VAN DEN HOEK, C. (1966). Isolation of living algae growing in the shells of molluscs and barnacles with EDTA (ethylenediaminetetraacetic acid). *Blumea*, **14,** pp. 331–332.

QUEKETT, J. (1852). *Lectures on Histology.* Elementary Tissues of Plants and Animals. Skeleton of mollusca–conchifera. Vol. 2, Lecture 16, pp. 276–278, London.

RANSON, G. M. (1936). "La maladie du pied" chez *Gryphaea angulata Lmk.* en 1935 dans la région de Marennes. *C. r. Séanc. Soc. Biol.*, **121,** pp. 540–542.

RANSON, G. (1951). Les Huîtres. Biologie—Culture. Paul Lechevalier, Paris.

RAY, S. M., MACKIN, J. G. and BOSWELL, J. L. (1953). Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum. Bull. mar. Sci. Gulf Caribb.*, **3,** pp. 6–33.

ROCHE, J., RANSON, G. and EYSSERIC-LAFON, M. (1951). Sur la composition des scléroprotéines des coquilles des mollusques conchiolines). *C. r. Séanc. Soc. Biol.*, **145,** pp. 1474–1477.

ROSE, C. B. (1855). On the discovery of parasitic borings in fossil fish-scales. *Trans. microsc. Soc. Lond.*, N.S. III, pp. 7–9.

SANDOZ, M. D., ROGERS, R. and CURTIS, L. N. (1944). Fungus infection of eggs of the blue crab *Callinectes sapidus* Rathbun. *Science*, **99,** pp. 124–125.

SANTESSON, R. (1939). Amphibious pyrenolichens I. *Ark. Bot.*, **29,** No. 10, pp. 1–67.

SCHAJOWICZ, F. and CABRINI, R. L. (1956). Chelating agents as histological and histochemical decalcifiers. *Stain Technol.*, **31,** No. 4, pp. 129–133.

SCHLOFSBERGER, J. (1856). Zur näheren Kenntnifs der Muschelschalen, des Byssus under der Chitinfrage. *Justus Liebigs Annln Chem.*, N.S., **22,** pp. 99–120.

SCHWARTZ, F. J. (1963). A new *Ichthyosporidium* parasite of the spot (*Leiostomus xanthurus*): a possible answer to recent oyster mortalities. *Progve Fish Cult.*, **25,** No. 4, pp. 181–186.

SIEBERT, W. (1913). Das Korperepithel von *Anodonta cellensis. Z. wiss. Zool.*, **106,** pp. 450–526.

SINDERMANN, C. J. (1968). Bibliography of oyster parasites and diseases. *Spec. Scient. Rep. U.S. Fish. Wildl. Serv.*, No. 563, 13 pp.

SINDERMANN, C. J. and ROSENFIELD, A. (1967). Principal diseases of commercially important marine bivalve Mollusca and Crustacea. *Fishery Bull. Fish. Wildl. Serv. U.S.*, **66,** No. 2, pp. 335–385.

SPARCK, R. (1926). A comparison between the Danish oyster beds and those in Holland and S.W. France.

Rep. Dan. biol. Stn, for 1925, No. 31, pp. 59–63.

SREEBNY, L. M. and NIKIFORUK, G. (1951). Demineralization of hard tissues by organic chelating agents. *Science*, **113,** p. 560.

STIRRUP, M. (1872). On shells of mollusca showing so-called fungoid growths. *Proc. lit. phil. Soc. Manchester*, **11,** Session 1871–72, pp. 137–138.

TOPSENT, E. (1887a). Sur les pretendus prolongement périphériques des Cliones. *C. r. Lebd. Séanc. Acad. Sci., Paris*, **105,** p. 1188.

TOPSENT, E. (1887b). Notes sur les Thallophytes marins perforants. *Mem. de la Soc. Linnéene de Normandie*, 1887, p. 297.

TRUEMAN, E. R. (1949). The ligament of *Tellina tenuis*. *Proc. zool. Soc. Lond.*, **119,** No. 3, pp. 717–742.

TRUEMAN, E. R. (1950). Observations on the ligament of *Mytilus edulis*. *Q. Jl microsc. Sci.*, **91,** No. 3, pp. 226–235.

TRUEMAN, E. R. (1951). The structure, development, and operation of the hinge ligament of *Ostrea edulis*. *Q. Jl microsc. Sci.*, **92,** No. 2, pp. 129–140.

VOISIN, P. (1931). La maladie des huîtres de Zélande. *Revue Trav. Off.* (*scient. tech.*) *Pcêh. marit.*, **4,** No. 2, pp. 221–222.

WALNE, P. R. (1966). Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. *Fishery Invest., Lond.*, Series 2, **25,** No. 4, pp. 1–53.

WEDL, C. (1859). Uber die Bedeutung der in den Schalen Acephalen und Gasteropoden vorkommenden Canale. *Sber. Akad. Wiss. Wien*, **33,** No. 28, pp. 451–472.

YONGE, C. M. (1960). *Oysters*. Collins New Naturalist Monograph, No. 18, London, 209 pp.

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